β-phenyl galactoside.

Nov. 10, 1947.

Sample from E E Snell (2 grams).

Test in comparison with lactose + galactose at .05% in T(m).
Add necessary growth factors.

		galactose (A)	lactose (B)	β-φ galactoside. (C)	β-φ + galactose. (D)
1	58-161	+ ++ ✓	− (±) ++	± ± −	+ + ++
2	Y87.	+ ±+ ✓	− ±. ++	− − ✓	+ + ++
3	ω-30.	− −	− −	− −	− −
4	ω-35	++ ++ ✓	± ±. ✓ ++	− − ✓	±+ + ++
5	ω-36.	++ ++ ✓	±. ± ✓ ±	− − ✓	− − ✓
7	Y10	+ ++ ++	++ ++ +++	− − + +±	± + ++
8	Y53.	++ ++ ✓	± ± + ✓	− − ✓	++ ++ ✓
6	ω-2.	± + ++	++ ++ ++	− − ± +±	±. ± ±

Readings at 20h., 24h., 36h.

φ-galactoside is not generally utilized and may be slightly inhibitory in galactose media. Cf Y10 however.

56 hours; 72h.

	gal	lac	β-φ gal	β-φ gal + gal.	
1	++	++	++ ✓	++	
2	++	++	− ✓	++	lac + cells present
3					
4	++	++	− ✓	++	
5	++	++	− ✓	++	
6	++	++	+± ✓ ✓	++	
7	++	++	++	++	
8	++	++	−	++	

Note that none of these cultures originally lac− have grown on β-φ-gala. Considerable pigment produced on galactose

Nov 15 1947

Inocula from 23 SP15. 0.1 ml/tube T(BMTLB1) base.

		A(Galactose .05%)	B (β-Φgalactoside)	C Galactose +Phenol .02%
TIME::::		SP16	SP16	SP16.
	Inoculum			
1	sb gal 1a	+++	++	++
2	161 lac 1b	+++	++	++
3	pb 1c	+++	+++	++
4	187 gal 2a	+++	−	++
5	lac 2b	+++	++	++
6	410 gal 7a	+++	±	++
7	lac 7b	+++	++	++
8	pb 7c	+++	+++	+++
9	gal 8a	+++	−	++
10	lac. 8b	+++	++	++

?? Is utilization of β-Φgalactoside by wild type mutants?

		SP17
on gentiobiose	+	+
"α-Φ-galactoside"	+	++
2a on gentiobiose	+	+
"α-Φ-galactoside"	+	++

P17. Streak out on β-Φglucoside EMB:

1A ; 1C., 1B.

6A; 6C.

A19. 1: all show a slow type of colony & a few much darker suggestive of rapid utilization. 1B and 1C show these particularly. all streaks are papillated.

6: somewhat smeared. Two colony types also noted.

Needs checking & phenol + galactose.

Nov. 27, 1947

Test on EMB agar using heavy water suspensions of cells from YP agar slants, except W-28 and W-29 from galactose EMB agar.
48 hr. readings.

		W33	+++	W35	-		
		W37	++	W36	-		
		W38	++	Y70	++		
1.	K12. ++	W41	++	W40	++	Y53	++
2	Y10 ++	W28	++	W42	++	Y87	++
3	58-161 ++	W29	++	W43	-	W30	++
4	W52 +++	W44	++	W45	-	W53	+
		W46	++	W48	-		
		W50	+±	W49	-		
		W51	++±	W-1	+++		

24 hrs. (A29) W52 + All others -

36 hrs. W52 +++ W-1, W33 ++, Y10 +, Y70, Y53 ± W53: -

48 hrs. 60 hrs. As above T.

There seems to be a graded spectrum of responses. Y52, W-1, W-51 and W-33 are distinctly the most positive reactors, especially W52. The "negative" types are all "sectorial" mutants derived from 58-161 and are Lac negative. Since their Lac+ counterpart is Bø+ a relationship is suggested! The only strain which is even relatively "Lac+/Bø-" is W53. While Y53 is Lac- Bø+.

Note: Lac+ Lac-

~~Lac~~ Bø+ Y10 Y53, W-1.

Bø- W53 W45, -49.

Suggested Crosses. W53 x W-1 Lac+ Bø- x Lac- Bø+, also Mal+/-

W49 x Y10 Lac- Bø- x Lac+ Bø+.

Action of all mixtures on lactose.

Trehalose / Maltose Cross adaptation. pulcini.

Dec. 10, 1947.

Prepare 10^{10} suspensions of

a. Y40 Lac+

b. W-1 Lac_1-

c. W-45 Lac_2-

Inc. in 37° water bath

Add 1 ml bacteria to 1 ml 4% lactose + dil. to 5 ml. Use Durham tube for gas, and BCP for acid production. Do mixtures in duplicate. + refers to acid production. (.1 ml M/10 buffer pH 1.0 added.)

		6P9	9 A10 P10	Set up 3:45 P9
1.	a	–	+++, +++	
2.	b.	–	–	
3.	c.	–	–	
4.	a+b	–	+++, +++	
5.	a+c	–	+++, +++	
6.	b+c.	–	–, –	
a	glycose	+++	+++	
c	glucose	++	+++	

Mixtures of Lac_1- and Lac_2- therefore cannot ferment lactose.

Adaptation takes some hours under these conditions. (No extra N)

Dec. 11.

For Trehalose, use culture of Exp 25 and compare ~~i~~ glucose adapted from same culture. (Controls are inadequate.) Set up 4:15 P 11.

	Grown in	Testing on
A	glucose.	glucose
B	"	maltose
C	Trehalose	glucose
D	"	maltose

TREHALOSE***MALTOSE CROSS-ADAPTATION EXPERIMENT.

Dec. 16, 1947.

Grow K-12 in T(0) plus .05% sugar 24 h. Harvest and concentrate to ca 10^{10} /ml/

Add 1 ml. cells to 1 ml 5% sugar, and in replicates add NaN_3 to a final conc. of 2×10^{-3} M. Add 0.1 ml M/10 phosphate buffer pH 7.0 and ,05 ml BromCresolPurple .15%

Make up to 5 ml with water, cells added 2 P 16, incubate in 37° water bath.

Readings at 2 h., 4 h., and 18 h., Readings - unless indicated.

Cellogenesis: 2h. 4h. 18h.
4P17 6P17 10A18

Set up. 2P17

A. Glucose
B. Maltose
C. Trehalose.
} T(0) + .05% sugar 18 hours. Harvest + concentrate.

A.	Gluc.		+++	+++
	" + Azide		±	+++
	M		−	+++
	" + Az		−	−
	Tr		−	+++
	" + Az		−	−
B.	G		+++	+++
	" + Az		−	+++
	M		++	+++
	" + Az		−	+++
	Tr		−	+++
	" + Az		−	−
C	G	+++	+++	+++
	G + Az		−	+++
	M		++	+++
	M + Az		−	+++
	Tr	±	++	+++
	Tr + Az		−	+++

A cells did not adapt in 18 hrs. in presence of azide, either to trehalose or to maltose.

B cells utilized maltose in the presence of azide, but did not adapt to trehalose.

C cells utilized maltose as well as trehalose and glucose, even in presence of maltose.

Azide in conc. of 2×10^{-3} M does inhibit fermentation to some extent but seems to block adaptation completely.

Conc. trehalose and maltose cross-adapt, but only unilaterally, trahalpse adaptation implying maltose adaptation, but not the converse.

Query: Will Malt-(Tre-) cells utilize maltose if grown on trehalose?

Azide does seem to interfere with the fermentation as well as adaptation.
Tr-adapted seem to be maltose adapted but not vice versa

The inhibition of lactose-adaptation by Azide.

Dec. 18, 1947.

Harvest K-12 from YP-.1%glucose broth. 16 hr. cultures. Conc. 50/20.

Tubes contain in 3 ml. , : 1% sugar, 1 ml cells, .1ml Phosphate Buffer M/10 pH 7.0 and indicated conc. azide or DNP Set up 12:20 PM

	Glucose Azide M/100 X	(3:20) 3:40PM.	6:00PM	21.h. 9A20.	Lactose 3:40	6:00PM.	7:00PM	21.h.-	(pH.
1.	—	+++	✓	✓ 4.50	-	+	++	+++	4.62
2.	1	++	✓	✓ 5.79	-	—	—	—	6.28
3.	.5	+±	++	✓ 5.57	-	—	-	+	5.95
4.	.1	+++	✓	✓ 4.78	-	±	+	++±	5.48
5.	.05	+++	✓	✓ 4.70	-	+	++	+++	5.18 ~~4.78~~
6	.01	+++	✓	✓ 4.36	-	+	++	+++	5.01 ~~5.18~~
	DNP 10^{-4}M x								
7	5	—	✓	✓	-		—		
8	1	++	✓	✓	-		—		
original solution									7.37

At 12:40, none changed.

DNP itself is an indicator. 10^{-3} Azide does not appreciably inhibit fermentation. but it does permit slight adaptation.

The pK of phosphate buffer is 7.21. $K = 6.2 \times 10^{-8}$ $pH = pK + \frac{(base)}{(acid)}$

At the initial pH the ratio is ca. 1.6 : 1 There are altogether 10 mM phosphate. At pH 4.50, the ratio is 1:50. The lower the pH, the more sensitive the pH is to slight additions of acid. → i.e. all but 2% of the base is neutd., and about 6 mM H^+ have been produced (from 30 mg = $\frac{1}{6}$ mM = 167 mM glucose). More buffer should be used in this system and an indicator used whose pK is near the pK of phosphate. such as bromthymol blue.

Dec. ~~18~~-18, 1947.

W-34.

Grow ~~[redacted]~~ in T(TC) + .1% trehalose and glucose. No growth (±) on maltose.

Test for activity on glucose and maltose in system like Exp. 65.

Harvest 50 ml & conc. to 2 ml. 50/2. Set Up. 5P19.

Growth in ↓ Action on →	2h. 7P19	Glucose 9A20	Maltose.
Glucose	+++	+++	– –
Trehalose.	+±	+++	– –

W-1 is therefore capable of producing trehalase but not maltase.

So far, all Mal- mutants are apparently Tre+, although W-21 is perhaps a little slow on trehalose.

Maltase is not simply an incidental activity of trehalase.

Cross-adaptation of galactosides

Jan. 14, 1948.

Harvest cells from .1% cultures in T(m) 36h. into 4/ml. (K-12)

Set up tests with 1 ml cells, 1 ml 3% substrate, M/200 Hazide and .1 ml M/10 phosphate. BCP indicator.

Substrates: G, glucose; L, lactose; M, b-methylgalactopyranoside; and B, N-Butyl-b-galactopyranoside., Ga, galactose.

Grown in/tested on: Set up 11A, 37°.

	G/GA	G/G	G/L	G/M	G/B	L/G	L/L	L/M	L/B	L/Ga
5PM	—	±	—	—	—	—	—	—	—	—
10A 15. (23h.)	—	+++	—	—	—	+±	++	+	—	±

	M/G	M/L	M/M	M/B	B/G	B/L	B/M	B/B
5PM	±	±	±	—	±	±	—	—
10A15 (23h.)	+++	+++	+++	±	+++	+++	+	±

Grown ↓ / Tested →	Glucose	Lactose	Butyl-gal.	Methyl-gal.	Galactose
Glucose	+++	—			—
Lactose	+±	++	—	+	±
Butyl--	+++	+++	±	+	
Methyl--	+++	+++	±	+++	

Cells probably too old for rapid adaptation. Lactose cells in experiment conditions.

In future, use mixture of BCP and BTB or most marked contrasts.

Use 2 BTB : 1 BCP. Cells may be too old.

Concl. ① M adapted are L adapted. ② L adapted are M adapted

③ B is poorly utilized under these conditions! ④ Galactozymase is adaptive

⑤

W108: Test for maltase and lactase activity.
Utilization of C-sources

Jan. 23, 1948.

Grow W-108, Y87, W56 and Y10 in YB broth overnight. Use ½ ml inocula into 10 ml. indicator broth with 1% sugar.

	Maltose			lactose			
108	—	—	—	—	—	++	adapted
108	—	—	—	—	—	++	
87	+++	✓	✓	—	—	—	much earlier
87	+++	✓	✓	—	—	—	
56	±	✓	✓	+++	+++	✓	but thin on
56	±	✓	✓	+++	✓	✓	
108;56	±	✓	✓				maltose.
108;56	±	✓	✓				
108;87				—	—	—	
108;87				—	—	—	
Y10	+++	✓	✓	+++	✓	✓	
Y10	+++	✓	✓	+++	✓	✓	

By P25 all +++ except W56/M..

Therefore, W108 cells do not produce maltase detectable by the utilization of the hexose components by symbiotic W56, and conversely with lactose and Y87.

Use small inocula from slant-suspensions. T(m) with .05% equiv. C-source.

W-108: Inoc. P23.

	N24.	P25	P28	
glucose	—	±	+++	→ M-L- Streak out on glu + trehalose.
fructose(st sep)	—	—	+++	→ M+L+
trehalose "	—	—	+++	→ M-L-.
sucrose	—	—	—	
maltose	—	++	+++	→ M+L+
lactose	—	—	—	
Na lactate	++	+++	✓	
K gluconate	+++	+++	✓	

Y-10 glucose +++ +++.

W108 Y10

On 1% EMB plates:

	N24.	P25	
K glucon	++	+++	+++
glucose	—	— many variants	+++
l-arabinose	+++	✓	+++
xylose	+++	✓	+++
mannitol	—	occ. pos.	++
lactose	—	"	+++
maltose	—	"	+++

Looks for specific phenotypic variants on glucose, maltose & lactose selections

W108: Test for β-galactosidase activity 94

Jan 26, 1948

Inoculearly W108 + Y10 into T (m) + .05% PΦgalac. & .05% K gluconate. Incubate 36 hours & test for free phenol with diazo-sulfanilic reagent. (BΦgal gives a strong color which, however, disappears in acid solution!). Compare with blanks, etc.:

	Test 1.	
1. Blank	−	−
2. Blank medium (BΦgal)	−	−
3. ΦOH .02%	++++	++++
4. 108 a	±	+
5. 108 b	++	+
6. Y10 c	±	+
7. Y10 d	++	+
8. Y10/gluconic only,	−	−

Not even nearly complete splitting by either Y10 or W108 under these conditions. Streak out 108 on lactose plate to assure non-reversion.

Some splitting is autom- ca. 10%.

Cross adaptation tests.

Jan 28-9, 1948

		a Glucose	b Galactose	c Gluconic	d l-arab	e d-xyl.	6.
Y10	A Glucose	++ ++ ±	± ± ±	− −	− −	−	−
Y10	B Galactose	++ +++ ++ +++	− − −	−	++ ++ ++	−	−
W108!	C Gluconic ac.	+++ +++	− − +++ +++	− −	− −	−	−
W108!	D l-arabinose	± ± +++	− − −	− −	− −	−	−
Y10	E l-arabinose	++ ++ +++	± ++ +++	− −	− ++ ++ +++	−	
W108!	F d-xylose.	± ± ++	− − −	− −	− −	± ++ +++.	

No ferment.

1 hour
2 hours
4 hours.

(1) Gluconic and galactose are adaptive. Also d-xylose and l-arabinose.

(2). D-arabinose is not fermented

(3). Galactose and arabinose cross-adapt bilaterally.

(4). The resting cell suspensions of W108! utilize glucose !!! (Repeat).

Cells grown overnight and harvested from YP broth 50ml + 1% sugar. Concentrate to 7ml. Use 1 ml cells, 1ml azide buffer + 1% sugar.

→ found to be mostly Glu+ mutants.

Cross-adaptation tests.

January 30, 1948.

Grown in: ↓	A / Glucose	B // Galactose	C / Gluconic	D // l-Arabinose	E HDP.
1. Y10 Glucose	+ ++	± +	− ±	− ±	− −
2. Galactose	± ++	± ++	+ ±	± +±	− −
3. Gluconic	+ ++±	± +	+ ++±	±	− −
4. l-Arabinose	± ++	± ++	− −	± ++	− −
5. —	−	± −	− −	− −	− −
W108	+	− −			
6. * Glucose	− −	− −	− −	− −	− −
	−	−	−		
7. * Galactose	± +	± ++	− −	+ ++	− −
	++	++	±		
8. Gluconic	− 8	− −	+ +++	−	− −
	±	±	+++		
9. * l-Arabinose	− ±	± ++	− −	+ +++	− −
	+	+++	±		
10. —	− −	− −	− −	− −	− −
	−	−	−		

may be too heavily buffered.

cells o.k.

cells o.k.

cells o.k.

galactose by W108 −

#.

##.

Design as above. Cells added 11:30 A.M. Variable cell yields!
Arside + phosphate.

2 h. 3½.

* streak out on maltose or glucose

(1). Confirm cross-adaptation of galactose & arabinose

~~(2). Glucose is adaptive. Glucokinase is lacking in gluconic adapted cells.~~

W108 - C source characterization

T(m) + .05% C source.

	Glucose	MDP	Gluc + MDP
W108	−	+±	+±
Y10.	+++	++	+++

24 hours.